Effects of a Microbial Enzyme Supplementation on the Performance of Laying Hens Fed Diets Containing Different Levels of Wheat

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ABSTRACT : An experiment was conducted to evaluate the effect of a microbial enzyme (Roxazyme-G®), a multi-carbohydrases preparation, supplementation to the wheat-based layer diets. Diets were formulated to include different levels of wheat replacing yellow corn on isocaloric and isonitrogenous basis. The energy value of wheat in the enzyme supplemented diets was adjusted (spec-modified) to have 5% more ME than the wheat in diets without enzyme. A total of 864 Hy-Line® brown layers were assigned to 4 dietary treatments: 10% wheat (T1), 25% wheat (T2), 25% wheat (spec-modified)+ 0.01% Roxazyme-G® (T3), and all wheat (spec-modified) +0.01% Roxazyme-G® (T4). Hen-day egg productions of T1 and T4 were significantly ($p < 0.05$) greater than that of T2 but not different from T3. Hen-housed egg production of T4 was significantly ($p < 0.01$) greater than those of T1 and T3 but not different from T2. Egg weights of T1 and T2 were significantly ($p < 0.01$) greater than that of T4. Feed consumption of T2 was significantly ($p < 0.01$) lower than other treatments. Feed conversion ratio (feed/egg mass) was not significantly different among treatments. Eggshell thickness of T1 was significantly ($p < 0.01$) greater than other treatments but ratio of broken eggs was not significantly different among treatments. Haugh unit of T4 was significantly greater ($p < 0.05$) than that of T2. Egg yolk color was significantly ($p < 0.01$) influenced by treatments in which enzyme treatment potentiated the yolk pigmentation. It was concluded that a multi-carbohydrases supplementation enables complete replacement of yellow corn with wheat without loss of productivity and major egg quality parameters.

(**Key Words**: Multi-carbohydrases, Wheat-based Diet, Layer Performance, Egg Quality, Yolk Pigmentation)

INTRODUCTION

Wheat is a major source of energy in livestock feeds, but it is not so frequently used as a sole source of grain in poultry diet in some part of the world, such as Korea, because of low content of carotenoids and the presence of a group of nonstarch polysaccharides (NSP) known as arabinoxylans (or pentosans). The NSP fraction is present in wheat at an appreciable level (5-8% of DM, Annison, 1991). Some studies show that wheat contains two fractions of pentosans, a water-extractable and an alkali-extractable fraction (Annison, 1990). The water-soluble pentosans are assumed to be primarily responsible for the anti-nutritive activities. Solublized pentosans in the small intestine of birds cause a sticky, viscous material to form. It is hypothesized that an increase in the bulk and viscosity of the intestinal contents decreases the rate of diffusion of substrates and digestive enzymes and hinders their interaction at the mucosal surface. Another hypothesis is that the viscous nature of these NSP might directly complex with digestive enzymes and reduce their activity (Schutte et al., 1993). As a consequence of anti-nutritional effect of NSP, digestibility of starch, fat, amino acids, vitamins, and minerals is reduced. Feed intake is also reduced. Rogel et al. (1987) found no evidence to suggest that viscous NSP within wheat inhibit its digestion, however.

The problems related to NSP can be overcome by using wheat at low levels (perhaps 10 to 20%) or by the use of suitable enzymes. Recent works conducted at various institutions have identified the primary mode of action of feed enzymes in wheat- or barley-based diets. The effect is on intestinal viscosity (Wyatt, 1995). Pettersson et al. (1991) reported that the enzyme supplementation diminished the high viscosity of the intestinal contents and reduced sticky droppings from the broilers fed pelleted diets based on barley, wheat and rye. Schutte et al. (1993) obtained similar results from a study that used a xylanase enzyme preparation in a wheat-based

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broiler diet. The viscosity of ileal digesta and water intake decreased and growth and feed conversion efficiency improved. Mollah et al. (1983) found that the AME_n and starch digestion of low ME Australian wheats were significantly correlated. Friesen et al. (1992) also reported that the enzyme supplementation may have increased the wheat AME_n through enhanced starch digestion and reduced variability among birds.

The objective of the present study was to determine if supplementation of microbial carbohydrase product to wheat-based layer diet can be a viable alternative to corn-based diet under practical condition.

MATERIALS AND METHODS

Experimental diets

The fromulation and composition of the experimental diets are shown in table 1. Wheat used in the

Table 1. Formula and composition of diets

Ingredients	Experimental diets (g/kg)[5]				
	Pre-lay	T1	T2	T3	T4
Corn, yellow dent, US No. 3	479.0	518.7	410.8	390.0	–
Wheat, Canadian	100.0	100.0	250.0	–	–
Spec-modified wheat	–	–	–	250.0	668.7
Soybean meal (44% CP)	135.0	158.0	155.0	153.0	94.0
Limestone	45.1	90.8	90.9	90.9	91.1
Full-fat soybean	–	–	–	–	52.1
Wheat bran	151.0	35.0	–	25.0	22.0
Rapeseed meal	30.0	30.0	30.0	30.0	30.0
Corn gluten	26.0	32.0	27.0	25.0	5.0
Animal fat	15.0	15.0	15.0	15.0	15.0
Calcium phosphate (18% P)	9.6	11.1	11.4	11.1	10.8
Layer premix[1]	5.0	5.0	5.0	5.0	5.0
Salt	2.9	3.2	3.2	3.2	3.2
DL-Methionine (50%)	1.4	1.2	1.6	1.7	3.0
Lysine-HCl (78%)	–	–	0.1	–	–
Roxazyme[2]	–	–	–	0.1	0.1
Carophyll Red[3]	–	–	+	+	+
Carophyll Yellow[4]	–	–	–	–	+
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Calculated analyses					
ME (MJ/kg)	11.51	11.51	11.51	11.51	11.51
Crude protein	160	160	160	160	160
Lysine	7.1	7.1	7.1	7.1	7.1
Methione + Cystine	6.2	6.2	6.4	6.4	6.9
Calcium	20.0	37.5	37.5	37.5	37.5
Available phosphorus	3.5	3.5	3.5	3.5	3.5
Total phosphorus	6.0	6.0	5.9	6.0	6.2
Linoleic acid	15.1	14.2	12.0	11.9	10.0

[1] Provides per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 0.25 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_6, 3 mg; vitamin B_{12}, 10 μg; niacin, 30 mg; pantothenic acid, 10 mg; folic acid, 1 mg; choline, 250 mg; ethoxyquin, 125 mg; I, 0.5 mg; Zn, 50 mg; Mn, 40 mg; Fe, 60 mg; Cu, 10 mg; Se, 0.2 mg.

[2] F. Hoffmann La Roche Ltd., CH4002 Basel, Switzerland.

[3, 4] Pigmenters produced by F. Hoffmann La Roche Ltd.; supplemented at the level of 5 mg/kg for Carophyll Red and 25 mg/kg for Carophyll Yellow.

[5] T1: 10% wheat, T2: 25% wheat, T3: 25% wheat (spec-modified)+0.01% Roxazyme-G and T4: all wheat (spec-modified)+0.01% Roxazyme-G.

experimental diets to replace yellow corn was feed-grade Canadian wheat. The source of enzyme was Roxazyme-G®, a multi-carbohydrases preparation from *Trichoderma viride*. It contained 11,150 U/g cellulase, 27,600 U/g glucanase and 37,150 U/g xylanase activity as determined by the manufacturer. In order to reflect anticipated improvement of energy utilization by enzyme supplementation, the energy value of wheat used in the formulation of diet T3 and T4 was adjusted and termed 'spec-modified wheat'. As a result reported by Roche (1992) claimed that Roxazyme-G® improve AME value of wheat by 7 to 8%, the ME value of spec-modified wheat was assigned to 13.18 MJ/kg which is 5% greater than ME value assigned to regular wheat (12.55 MJ/kg). Carophyll Red® and Carophyll Yellow® were used as pigmenters in wheat-based diets. The nutritional composition of pre-lay diet fed for 2 wk pre-experimental period was same as T1 diet except the level of calcium. In the diet of T4, a restriction to minimum requirement for linoleic acid (1.0%) was applied in formulation.

Experimental design and feeding

Eight hundred and sixty four Hy-Line® brown pullets of 18 wk of age were assigned to four treatments based on the completely randomized block design. Each treatment was consisted of 12 replications of 9 cages (2 birds per cage) each. The four treatments were 10% wheat (T1), 25% wheat (T2), 25% wheat (spec-modified) +0.01% Roxazyme-G® (T3), and all wheat (spec-modified)+0.01% Roxazyme-G® (T4). T2 and T3 diets were supplemented with 5 mg/kg of Carophyll Red® and T4 diet with 5 mg/kg of Carophyll Red® and 25 mg/kg of Carophyll Yellow®.

Following 2 wk of pre-lay diet period, birds were given experimental diets for 21 wk. During the feeding trial, diets were presented in mash form and feed and water were given *ad libitum*. The house was provided with programmed lighting and ventilation. The lighting program started with 13 h of light on the initiation of experiment and was increased by 15 min every 2 wk until 17 h of light was achieved.

Measurement of parameters and data analyses

The number of eggs and egg weight were recorded daily. Feed consumption was measured weekly. Egg cleanliness was also recorded daily by visual score from 1 to 4 (very clean, clean, dirty, and very dirty). Specific gravity of eggs was determined three times a week using saline flotation method described by Hempe et al. (1988). Random samples of 24 eggs from each treatment were collected weekly to measure egg quality such as egg yolk color score (Roche color fan), eggshell thickness and Haugh unit. Body weight of birds was recorded at the initiation of lay (20 wk), 30 wk, and 40 wk of age. The data obtained from the experiment were analyzed by split block design to consider the effects of location of cages and laying period in weeks. General Linear Models (GLM) procedure of SAS® (SAS Institute, 1985) was used and significant differences between treatment means were determined using the Duncan's multiple range test option.

RESULTS

Overall performance of the birds fed experimental diets are shown in table 2. Hen-day egg productions of T1 and T4 were significantly ($p < 0.05$) greater than that of T2 but not different from that of T3. Hen-housed egg production of T4 was significantly ($p < 0.01$) greater than those of T1 and T3 but not different from T2. Egg weights of T1 and T2 were significantly ($p < 0.01$)

Table 2. Overall performance of laying hens fed experimental diets during 20 to 40 wk of age

Parameters	Treatments[1]				SEM
	T1	T2	T3	T4	
Egg production (%, hen-day)	74.65^{a}	73.60^{b}	74.03^{ab}	74.78^{a}	0.33
Egg production (%, hen-housed)	67.10^{BC}	67.67^{AB}	66.23^{C}	68.72^{A}	0.33
Egg weight (g)	58.73^{A}	58.65^{A}	58.51^{AB}	58.14^{B}	0.119
Feed consumption (g/hen/d)	128.83^{A}	126.35^{B}	129.05^{A}	129.23^{A}	0.339
Feed conversion (feed/egg mass)	2.93	2.92	2.97	2.95	0.039
Mortality (%)	7.84	6.05	7.84	6.38	1.143

[1] T1: 10% wheat, T2: 25% wheat, T3: 25% wheat (spec-modified)+0.01% Roxazyme-G and T4: all wheat (spec-modified)+0.01% Roxazyme-G.

$^{a-b, A-C}$ Means with different superscript in the same row are significantly different at $p < 0.05$ (a-b) and $p < 0.01$ (A-C).

greater than that of T4 but not different from that of T3. Feed consumption of T2 was significantly ($p < 0.01$) lower than other treatments. Feed conversion ratio (feed/egg mass) and mortality were not significantly different among treatments.

Table 3 shows the body weight change of the birds

Table 3. Body weight of laying hens fed experimental diets at 20, 30, and 40 wk of age

Age (wk)	Treatments[1]				SEM
	T1	T2	T3	T4	
20 wk	1,479.8	1,416.4	1,489.2	1,454.2	47.71
30 wk	2,001.3	1,909.8	2,001.7	1,965.5	54.59
40 wk	2,272.2	2,195.8	2,206.0	2,274.4	85.70
Weight gain (20 to 40 wk)	792.4	779.4	716.8	820.3	88.92

[1] T1: 10% wheat, T2: 25% wheat, T3: 25% wheat (spec-modified)+0.01% Roxazyme-G, T4: all wheat (spec-modified)+0.01% Roxazyme-G.

Table 4. Quality of eggs laid by hens fed experimental diets during 20 to 40 wk of age

Parameters	Treatments[1]				SEM
	T1	T2	T3	T4	
Specific gravity	1.0915	1.0914	1.0918	1.0918	0.0003
Eggshell thickness (μm)	410.81^{A}	405.43^{B}	404.18^{B}	403.04^{B}	1.163
Broken egg ratio (%)	0.69	0.73	0.64	0.63	0.111
Haugh unit	92.1^{ab}	91.8^{b}	92.6^{ab}	93.0^{a}	0.334
Egg cleanliness[2]	1.24	1.23	1.25	1.22	0.028
Egg yolk color score[3]	8.5^{C}	8.8^{B}	10.2^{A}	6.7^{D}	0.045

[1] T1: 10% wheat, T2: 25% wheat, T3: 25% wheat (spec-modified)+0.01% Roxazyme-G, T4: all wheat (spec-modified)+0.01% Roxazyme-G.
[2] Visual score from 1 to 4 (very clean, clean, dirty and very dirty).
[3] Measured by Roche color fan.
[a-b, A-D] Means with different superscript in the same row are significantly different at $p < 0.05$ (a-b) and $p < 0.01$ (A-D).

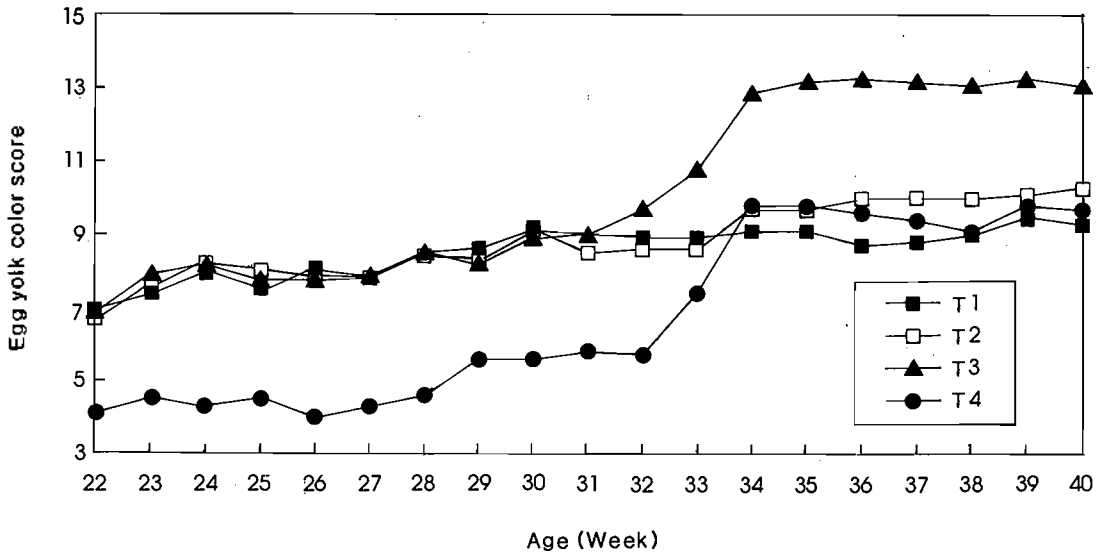

Figure 1. Yolk color score of eggs from birds fed experimental diets. T1 = 10% wheat; T2 = 25% wheat; T3 = 25% wheat (spec-modified)+0.01% Roxazyme-G; T4 = all wheat (spec-modified)+0.01% Roxazyme-G.

during the experimental periods. Birds of T4 gained most and those of T3 gained least but they were not different significantly.

Results of measurements to determine quality of eggs are shown in table 4. Specific gravity of eggs was not significantly different among treatments. Eggshell thickness of T1 was significantly ($p < 0.01$) greater than other treatments but ratio of broken eggs was not significantly different among treatments. Haugh unit of T4 was significantly ($p < 0.05$) greater than that of T2 but not different from T1 and T3. Egg cleanliness were not significantly different among treatments. Overall egg yolk color score of T3 was significantly ($p < 0.01$) greater than other treatments. Weekly change of egg yolk color score (figure 1) showed that T1, T2, and T3 were not significantly different but T4 was much lower than others up to 32 wk of age. Egg yolk color score of T3 and T4 increased sharply after 32 wk of age and reached the plateau at 34 wk of age. After 32 wk of age, T3 showed higher score than other treatments, which were not much different among themselves. The score of T2 also increased slightly after 33 wk but the extent of the increase was not as great as those of T3 or T4.

DISCUSSION

Overall performance of the birds fed experimental diets indicates that wheat can replace yellow corn in the layer diet if formulated iso-caloric and iso-nitrogenous and supplemented with a proper multi-carbohydrases product. For the complete replacement of corn with wheat in diet formulation, pigmentation of yolk and the requirement for linoleic acid should be considered because wheat is lower in pigments and linoleic acid content than yellow corn. By meeting linoleic acid requirement and with supplementary enzyme, wheat-based diet (T4) tended to show better egg production than corn or corn plus wheat-based diets. Lighter egg weight of T4 might be the result of higher egg production.

It is interesting to note that eggshell thickness of T2 (25% wheat diet) and especially treatments of enzyme supplementation (T3 and T4) are significantly lower than T1 (corn-based diet). It is known that wheat has a significant phytase activity (700 U/kg) but corn does not (Pointillart, 1993). It is postulated that phytase P in wheat-based diets became more available due to the activity of endogenic phytase in wheat and possibly to supplementary enzyme to some extent. High level of available P can cause a decrease of plasma Ca available for shell formation. If the above postulation is verified, it would be necessary to adjust the value of available P of wheat when supplementary enzyme is used in diet formulation. The reason for the significantly higher Haugh unit of enzyme treatments (T3 and T4) compared to T2 is not clarified in the present study but may warrant further investigation. Sticky droppings may not have influenced cleanliness of eggs probably because the birds were kept in cages. Higher color score of T3 than of T2 suggests that enzyme supplementation may increase absorption of pigmenters resulting in improved pigmentation efficiency.

It was reported that enzyme supplementation to wheat-based diet decreases amount of excreta (Wyatt, 1995). Because environmental pollution from animal manure becomes a worldwide concern, environment friendly animal feeding system is becoming more important. In this context, enzyme preparations for dietary supplementation may have an extra value in feed and animal industry.

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